

Transmission of Hepatitis C Virus in Taiwan: Prevalence and Risk Factors Based on a Nationwide Survey

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A nationwide community-based survey on hepatitis C virus (HCV) was carried out in seven townships in Taiwan. A total of 11,904 men aged 30–64 years were recruited for testing for antibodies against HCV (anti-HCV) by second-generation enzyme immunoassay. A total of 272 seropositive cases and 282 seronegative controls were interviewed to explore risk factors for HCV infection in the study areas. Spouses of 214 seropositive cases were identified to assess the concordance of seropositivity of anti-HCV between spouses; genotypes of HCV were also tested in 26 couples who were both seropositive. A significant geographic variation in seroprevalence of anti-HCV was observed in the study townships (1.6–19.6%). Blood transfusions, medical injections, acupuncture and tattooing were related to an increased anti-HCV seroprevalence showing multivariate-adjusted odds ratios of 8.6, 2.5, 3.1, and 2.2, respectively, with corresponding population attributable risk percentages of 25%, 57%, 16%, and 3%, respectively. The anti-HCV prevalence in spouses of index cases (24%) was significantly higher than that observed in the general population of the study areas (4%). However, a striking interspousal discrepancy in HCV genotypes (20/26 = 77%) was observed among both seropositive couples. Common exposures to medical injections and acupuncture were reported by 15 (58%) of these couples. This study identified some endemic areas of HCV infection in Taiwan. Iatrogenic factors were common vehicles for HCV infection, and a concordance of anti-HCV seropositivity between spouses may primarily be due to extrafamilial iatrogenic infectious sources in study areas. *J. Med. Virol.* 59:290–296, 1999.

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INTRODUCTION

Hepatitis C virus (HCV) has been identified as the major cause of post-transfusion hepatitis and sporadic non-A, non-B hepatitis [Alter et al., 1989, 1992] since its discovery in 1989 [Choo et al., 1989]. In addition, HCV infection leads to chronic liver disease such as cirrhosis of the liver, and eventually hepatocellular carcinoma [Kiyosawa et al., 1990]. Since the availability of the first-generation tests for antibodies against HCV (anti-HCV), the prevalence of HCV infection in healthy adults in Taiwan has been reported to range from 0.6% to 2.5% [Chen et al., 1990; Lee et al., 1991a; Wu et al., 1991]. However, these studies are subject to limitations of the low sensitivity and specificity resulting from the use of first-generation reagent or because of inadequate statistical power due to the small number of subjects. In addition, there has never been a systematic epidemiological study on HCV infection rates and transmission routes for the general population in the community setting in Taiwan. Thus, a nationwide community-based epidemiological study was carried out to examine HCV infection rates and to identify risk factors associated with community-acquired infection in Taiwan.

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MATERIALS AND METHODS

Study Area

Seven townships, including Sanchi, Chutung, Potzu, Kaohsu, Makung, Huhsi, and Paihsa, were selected in this nationwide survey on seroprevalence of anti-HCV in Taiwan. The former four townships are located in Taiwan Island, and the latter three are located in Penghu Islets. These seven townships were selected in order to compare anti-HCV seroprevalence between northern (Sanchi, Chutung) and southern (Potzu, Kaohsu) townships in Taiwan Island, as well as between townships with high (Makung, Huhsi, and Paihsa) and low (Sanchi, Chutung, Potzu, and Kaohsu) mortality rates from primary liver cancer. A total of 13 villages in Sanchi, 25 in Chutung, 27 in Potzu, 19 in Kaohsu, 34 in Makung, 22 in Huhsi, and 15 in Paihsa were studied.

Community-Based Seroprevalence Survey

All male residents who were 30–64 years old and who live in the study townships were invited to participate in this study on a voluntary basis. There were 47,079 male eligible individuals who were invited by letter to participate. As a consequence, a total of 11,904 (25.3%) men including 707 in Sanchi, 2,445 in Chutung, 1,882 in Potzu, 2,232 in Kaohsu, 3,300 in Makung, 788 in Huhsi, and 550 in Paihsa enrolled in this seroprevalence survey, and the informed consent was obtained from all participants.

Case-Control Study on Correlates of HCV Infection

To investigate risk factors for HCV infections, a matched case-control study of seroprevalent and seronegative individuals was carried out. Case subjects consisted of 272 HCV-positive individuals identified from five study townships including Potzu, Kaohsu, Makung, Huhsi, and Paihsa. The control group included 282 seronegative individuals, matched by age at enrollment and residential township to the HCV-positive cases.

Study on Transmission of HCV Between Spouses

Spouses of 240 anti-HCV-positive individuals were recruited from the study townships for the study on the concordance of HCV infection between spouses. All subjects participated in this component of the study on a voluntary basis with an informed consent. A total of 214 (89%) spouses agreed to participate. Furthermore, 26 spouse pairs, who were both positive for anti-HCV were examined for HCV genotypes.

Personal Interview and Blood Collection

Both personal interviews based on a structured questionnaire and blood collection were carried out by trained public health nurses at recruitment of study subjects. Information obtained from the questionnaire interview at baseline recruitment included age, ethnic-

ity, marital status, and educational levels. Questionnaire information obtained for case-control studies on correlates of infection included history of blood transfusion, hemodialysis, tattooing, and injections for medical treatment, or for vitamin supplements, glucose, and nutrients by the intradermal, intramuscular, and intravenous routes.

Blood samples were collected from each study subjects using a set of disposable needle and heparinized vacuum syringe. Plasma, buffy coat and red blood cells were separated and stored at -70°C in local epidemiological research units of this study. Biospecimens were sent on dry ice to the central laboratory in Taipei every 2 weeks, stored at -86°C until testing.

Laboratory Assays

Serum aliquots were tested for anti-HCV by enzyme immunoassay using second-generation commercial kits (Abbott Laboratories, North Chicago, IL). All samples with positive or borderline results were retested. Only repeatedly positive samples were considered anti-HCV positive. For HCV genotyping, RNA was extracted from 100 μL of serum by a modified acid-guanidine phenol chloroform method [Chomzynski et al., 1987] and cDNA was synthesized by reverse transcription (RT) with random primers [SuperScript, Gibco-BRL, Gaithersburg, MD]. Viral specific cDNA was amplified by polymerase chain reaction (PCR) method using primers designed from the putative core region as previously described [Okamoto et al., 1992]. The PCR amplicon was further typed by second PCR using four sets of type-specific primer pairs. Genotypes were classified according to the Simmonds classification [Simmonds et al., 1993]; the genotype was classified as unknown type if PCR products hybridized with consensus probe, but with none of the type-specific probes.

Statistical Methods

Both age-specific and age-adjusted seroprevalences (using the 1976 world population as the standard population) of anti-HCV were compared at the township level. Mantel-Haenszel chi-square tests were used to examine the statistical significance of the difference in age-adjusted seroprevalence rates in different areas. In the analyses of correlates of HCV infection, matched odds ratios (OR) and 95% confidence intervals (CI) were calculated to examine the magnitude of association between anti-HCV positivity and risk factors. Multiple conditional logistic regression analysis was used to estimate multivariate-adjusted ORs and their 95% CIs for various predictors. In the case-control analysis, population attributable risk percentage was derived for relevant risk factors based on their multivariate-adjusted ORs and exposure prevalence of relevant risk factors in the general population as estimated from the control group.

RESULTS

Table I shows the age-specific anti-HCV seroprevalence among study subjects in seven townships. In gen-

TABLE I. Seroprevalence of Antibodies Against Hepatitis C Virus Among Residents in Seven Townships in Taiwan

Township	No. of subjects	Age-specific prevalence (%)				Age-adjusted ^a prevalence (%)
		30–39	40–49	50–59	60–64	
Taiwan Island						
Sanchi	707	4.3	12.5	13.6	14.7	10.3
Chutung	2,445	1.8	1.9	5.2	3.2	2.8
Potzu	1,882	6.0	6.6	6.9	8.2	6.7
Kaohsu	2,232	0.9	1.7	1.9	1.8	1.5
Penghu Islets						
Makung	3,300	1.5	0.7	1.9	2.0	1.4
Huhsi	788	5.2	2.4	3.6	4.6	3.9
Paihsa	550	11.3	20.0	23.7	17.7	17.8

^aThe 1976 world population was used as the standard population for age adjustment.

TABLE II. Multiple Logistic Regression Analysis of Associations Between Sociodemographic Characteristics and Seroprevalence of Antibody Against Hepatitis C Virus Among Residents in Seven Study Townships in Taiwan: 1991–1992

Sociodemographic characteristics	Group	No. of subjects	Prevalence (%)	Multivariate-adjusted odds ratio (95%CI)
Residential township	Makung	3,300	1.6	1.0 (referent)
	Huhsi	788	4.1	4.8 (3.3–7.0)*
	Paihsa	550	19.6	14.7 (9.8–22.1)*
	Sanchi	707	10.9	7.6 (5.1–11.5)*
	Chutung	2,445	3.0	1.0 (0.6–1.7)
	Postzu	1,882	6.8	1.9 (1.0–3.8)
	Kaohsu	2,232	1.7	2.5 (1.5–4.1)*
Age (years)	30–39	3,422	2.9	1.0 (referent)
	40–49	2,871	3.7	1.1 (0.8–1.6)
	50–64	5,611	5.4	1.4 (1.1–1.9)*
Ethnicity	Fukienese	7,787	5.0	1.0 (referent)
	Hukka	3,130	2.8	1.2 (0.6–2.1)
	Others	861	2.8	0.6 (0.4–1.0)
Marital status	Married	11,220	4.3	1.0 (referent)
	Unmarried	684	4.1	1.2 (0.8–1.9)
Years of schooling completed	0	1,151	7.4	1.0 (referent)
	1–6	5,073	4.8	0.8 (0.6–1.0)
	7+	5,564	3.2	0.7 (0.5–0.9)

* $P < 0.05$.

eral, there was an increasing trend in prevalent infections with age. A striking difference in anti-HCV seroprevalence was observed among seven townships. After adjustment for age, study subjects in Paihsa (17.8%) and Sanchi (10.3%) had the highest age-adjusted seroprevalence, and those in Makung (1.4%) and Kaohsu (1.5%) had the lowest.

Multiple logistic regression analysis was carried out to examine the associations between anti-HCV seroprevalence and various sociodemographic characteristics. As shown in Table II, residential township, age and educational levels (indicated by schooling years completed) were all significantly associated with anti-HCV seropositivity. Compared with residents who live in Makung as the referent group, the multivariate-adjusted ORs (95%CI) were 4.8 (3.3–7.0), 14.7 (9.8–22.1), 7.6 (5.1–11.5), and 2.5 (1.5–4.1), respectively, for those who live in Huhsi, Paihsa, Sanchi, and Kaohsu. In addition, anti-HCV seroprevalence was found to increase with advancing age, and decrease with increasing schooling years completed. No significant association with anti-HCV seroprevalence were observed for

ethnicity and marital status after multivariate adjustment.

Results from the case-control analysis of association with HCV infection for iatrogenic risk factors and tattooing are shown in Table III. The history of blood transfusion (OR = 8.6, 95%CI = 4.5–6.4), medical injection (OR = 2.5, 95%CI = 1.7–3.6), and acupuncture (OR = 3.1, 95%CI = 1.8–5.2) were significantly associated with the seropositivity of HCV. Whereas, the association for tattooing was not statistically significant. There were only two seropositive cases and none of seronegative controls with a history of hemodialysis; these two cases with the experience of hemodialysis also reported a history of medical injection. In the multiple conditional logistic regression analysis after adjustment for residential areas, age, and educational levels, history of blood transfusion, medical injection, and acupuncture remained significantly associated with anti-HCV seropositivity. In addition, the population attributable risk fractions for blood transfusion, medical injection, acupuncture and tattooing were 25.3%, 57.3%, 15.7%, and 3.2%, respectively (Table IV).

TABLE III. Case-Control Study on Associations With Hepatitis C Virus Infection for Iatrogenic Risk Factors and Tattooing

Risk factor	Category	Cases (n = 272)		Controls (n = 282)		Odds ratio (95%CI)	
		No.	(%)	No.	(%)		
Blood transfusion	No	199	(73.2)	268	(90.1)	1.0	(referent)
	Yes	73	(26.8)	11	(3.9)	8.6	(4.5–6.4)*
Hemodialysis	No	270	(99.3)	276	(100.0)	1.0	(referent)
	Yes	2	(0.7)	0	(0.0)	5.1 [†]	(0.2–10.7)
Medical injection	No	65	(23.9)	122	(43.9)	1.0	(referent)
	Yes	207	(76.1)	156	(56.1)	2.5	(1.7–3.6)*
Acupuncture	No	215	(79.0)	259	(92.2)	1.0	(referent)
	Yes	57	(21.0)	22	(7.8)	3.1	(1.8–5.2)*
Tattooing	No	265	(97.4)	277	(98.9)	1.0	(referent)
	Yes	7	(2.6)	3	(1.1)	2.2	(0.6–8.1)

* $P < 0.05$.[†]Calculated by the exact method.

TABLE IV. Multivariate Adjusted Odds Ratios and Population Attributable Risk Fraction of Iatrogenic Risk Factors and Tattooing in Relation to Hepatitis C Virus Infection

Risk factor ^a	Comparison	Multivariate-adjusted ^b odds ratio (95%CI)	Population attributable risk fraction
Blood transfusion	Ever vs. never	8.6 (4.3–16.9)*	25.3%
Medical injection	Ever vs. never	2.4 (1.6–3.5)*	57.3%
Acupuncture	Ever vs. never	2.4 (1.4–4.2)*	15.7%
Tattooing	Ever vs. never	3.1 (0.7–13.3)	3.2%

^aHemodialysis not included in the regression model due to no controls had the history.^bOdds ratios were also adjusted for residential township, age, and years of schooling.* $P < 0.05$.

TABLE V. Seroprevalence of Antibodies Against Hepatitis C Virus Among Spouses of Seropositive Individuals in Comparison With General Population in Study Townships

Township	Anti-HCV positivity ^a	
	Spouses of seropositives (%)	General population (%)
Potzu	33.3	6.8
Paihsa	25.2	19.6
Huhsi	14.3	4.1
Kaohsu	8.3	1.7
Makung	6.3	1.6
Total	24.0	4.1

^aSeroprevalence in spouses was significantly ($P < 0.001$) higher than that in the general population.

As shown in Table V, the overall seroprevalence of anti-HCV in spouses of anti-HCV-positive index cases was 24%, which was statistically significantly ($P < 0.001$) higher than that in the general population of the same study areas (4.1%). The HCV genotypes of 26 couples, who were both seropositive for anti-HCV, are shown in Table VI. Thirty-two (61%) of these 52 individuals were infected by only one HCV genotype, two different genotypes were identified in 17 (33%) subjects, while genotypes for 3 (6%) individuals could not be determined. The overall prevalence rate demonstrated the highest proportion of type 2a (63% = 33/52), followed by type 1b (54% = 28/52) and type 2b (10% = 5/52). Among 26 couples studied, the HCV genotypes were the same in 15% (4/26) of couples and different in 31% (8/26) of couples infected with single genotype of HCV. In addition, there were 11 (54%) couples with one infected by only one HCV genotype

TABLE VI. Distribution of HCV Genotypes in 26 Couples Seropositive for Antibodies Against Hepatitis C Virus

Couple no.	HCV genotype Husband/Wife	Concordant iatrogenic Transmission routes
1	2a,2b/1b,2b	Unidentified
2	1b,2a/1b,2b	Medical injection
3	1b,2a/1b,2b	Unidentified
4	1b,2a/2a	Medical injection
5	2a/1b,2a	Unidentified
6	2a/1b,2a	Unidentified
7	1b/2a,2b	Medical injection
8	1b,2b/2a	Unidentified
9	1b,2a/1b	Medical injection
10	1b,2b/2a	Medical injection
11	1b,2a/2a	Medical injection
12	2a/1b,2a	Unidentified
13	2a/1b,2a	Medical injection
14	1b,2a/2a	Medical injection
15	1b/2a	Unidentified
16	1b/2a	Unidentified
17	1b/2a	Injection, acupuncture
18	1b/2a	Medical injection
19	1b/unknown ^a	Medical injection
20	1b/unknown	Medical injection
21	2a/1b	Unidentified
22	Unknown/2a	Medical injection
23	2a/2a	Unidentified
24	1b/1b	Medical injection
25	1b/1b	Unidentified
26	2a/2a	Injection, acupuncture

^aUnknown type indicates PCR product hybridized with the consensus probe, but with none of type-specific probes.

and another by two genotypes. All three couples who were both infected by two HCV genotypes had different HCV genotypes between husband and wife.

In the personal interview based on a structured

questionnaire to identify possible concordant HCV transmission routes between spouses, 15 (58%) couples reported common exposures to medical injection and acupuncture, while the remaining 11 couples had exposures with unidentified sources of infection.

DISCUSSION

The prevalence of HCV, derived from blood donor studies, is approximately 1% in many parts of the world [Jenny-Avital, 1998], while HCV-endemic areas have also been reported in the literature [Hara et al., 1996; Lu et al., 1997; Noguchi et al., 1997]. In this large-scale community-based study, a striking geographic variation in seroprevalence rates of HCV infection was observed among the townships, indicating the existence of common mode of transmission in areas with high infection rate. More importantly, some endemic areas of HCV infection have been identified in this study. Residents living in Paihsa and Sanchi Township had an extraordinarily high seroprevalence of anti-HCV, which was 10–20 times higher than the seroprevalence of 1% among blood donors in Taiwan [Chen et al., 1990]. Accordingly, studies in endemic foci of HCV would provide insights into the mechanisms of transmission.

It is interesting to note that age patterns of HCV antibodies varied widely among study townships. Overall, the prevalence of anti-HCV consistently increased with age in areas with a high prevalence of infection (i.e., Sanchi, Potzu, and Paihsa). By contrast, there was a nonuniform age trend of HCV seropositives in areas of low endemicity (i.e., Chutung, Kaohsu, Makung, and Huhsi). The explanation for the observed different age patterns of HCV antibodies among the townships is unknown. One possibility is that people living in communities in which inhabitants had high rates of HCV infection may frequently be exposed to a common reservoir of infection with efficient transmissibility (i.e., unsterilized syringes and needles frequently contaminated with HCV due to a high background rate of infection). Furthermore, since the major consequences of HCV infection are related to chronic infection [Sharara et al., 1996], the accumulation of HCV chronic infections coupled with efficient transmission could give rise to a uniformly increasing pattern of HCV seropositives with age in endemic areas. Further exploration of factors accounting for the observed different age patterns of HCV infection between endemic areas and those with low levels of infection is needed in order to develop strategies of curtailing the transmission of HCV in different community settings.

It was established in earlier studies of patients with non-A, non-B hepatitis, blood transfusion recipients, intravenous drug users and health care workers that HCV is efficiently transmitted by the parenteral route [Chen et al., 1990; Donahue et al., 1991; MacDonald et al., 1996]. Studies of community-acquired hepatitis conducted by the Centers for Disease Control and Prevention in the United States also elicited the presence of parenteral risk factors in 59% of cases [Alter et al.,

1992]. However, up to 40–50% of patients with HCV infection have no history of an identifiable parenteral exposure [Cuthbert, 1994], and there are likely to be unrecognized routes of transmission in these patients. As HCV infection is traditionally transmitted via the blood, post-transfusion hepatitis due to HCV infection accounts for the high rates of hepatitis in this setting [Alter et al., 1989; Esteban et al., 1990; Aach et al., 1991; Lee et al., 1991b; Watanabe et al., 1994]. However, since the implementation of specific testing for anti-HCV in donated blood, transmission by blood products was effectively reduced [Donahue et al., 1992; Wang et al., 1994, 1995]. Our case-control study showed that a history of blood transfusion was related significantly to anti-HCV seropositivity, as has been documented in previous studies of blood transfusion recipients [Alter et al., 1989; Esteban et al., 1990; Aach et al., 1991]. However, this finding must be interpreted with caution because the temporal relationship of blood transfusions with the acquisition of HCV was not measured directly in the present study. Moreover, only 27% of the antibody-positive cases had a history of blood transfusion, suggesting that additional risk factors may be involved in the acquisition of HCV infection in our study areas.

The data presented in this study suggest that iatrogenic injections account for HCV transmission in the townships, with a population attributable fraction of 57.3%. This finding was in accordance with previous reports that an increased risk of HCV infection was related to various types of medical treatment involving injections [Chen et al., 1995; Ho et al., 1997; Noguchi et al., 1997]. Injection with unsterilized syringes and needles was common in Taiwan before 1980 when disposable syringes and needles were not used universally. In Taiwan, a number of people believe that the medical injection is more effective and rapid than oral medications for treatment. In addition, some people are used to receiving glucose nutrient and vitamin injections so as to enhance their physical well-being. Thus, people with frequent exposures to the use of unsterilized syringes and needles could have an increased seroprevalence of anti-HCV similar to that of intravenous drug abusers, in whom the efficient transmission of HCV by needle stick and the high proportion of chronically infectious cases as a reservoir of infection have been documented [Chen et al., 1990; van den Hoek et al., 1990; Thomas et al., 1995]. Given these observations, it is essential to formulate a preventive strategy to interrupt the identified transmission route by iatrogenic injections.

HCV exists as multiple distinct genotypes and the distribution of virus genotypes could vary in different risk groups [Bukh et al., 1995; Pawlotsky et al., 1995]. Although the stability of HCV genotypes over long periods of time is not well documented in the literature, HCV genotyping has been used to identify the source of infection and the route of transmission in epidemiological investigations [Pawlotsky et al., 1995; Esteban et al., 1996; Noguchi et al., 1997]. Accordingly, the HCV

genotype was determined to evaluate the HCV transmission between spouses in the study. While the question of whether HCV is transmitted sexually is still unsettled, some investigators have obtained epidemiological data suggesting that sexual/household transmission occurs, albeit infrequently [Kao et al., 1992; Rice et al., 1993; Kao et al., 1996; Dienstag, 1997]. Intrafamilial transmission has been considered to play an important role in the observed familial aggregation and spouse concordance in HCV infection in Taiwan [Kao et al., 1992; Chang et al., 1994; Ho et al., 1994]. However, common exposure to extrafamilial sources in the community for family members may also result in familial aggregation and spouse concordance of the infection. According to our results, an excess in the seroprevalence of anti-HCV among spouses of anti-HCV-positive individuals was observed. However, most couples who were both positive for anti-HCV had different HCV genotypes in this study. As these couples have been married for at least 15 years, they should be concordant for HCV genotypes if sexual transmission were an important route in the spread of HCV between spouses. These findings suggest that sexual transmission of HCV may be relatively infrequent or less efficient in the study areas. Indeed, the excess in seroprevalence among spouses of seropositive individuals and the discrepancy in HCV genotypes between spouses may be due primarily to infection by common extrafamilial sources in the community by blood transfusion, medical injections, or acupuncture.

In conclusion, the data presented are consistent with the notion that iatrogenic injections involved in the spread of HCV. The use of unsterilized syringes and needles may explain HCV infection in the community in Taiwan. Given the lack of an HCV vaccine, interruption of transmission routes by iatrogenic injections with current technology and education of the general public are believed to be the main approach to prevent this infection.

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